

Claim Amendments & Complete Listing of the Claims

1. (Currently amended) A process for the production of egg yolk antibodies binding to small molecule organo chlorine pesticides, the said process comprising the steps of:

- (a) selecting a suitable from poultry bird; birds;
- (b) immunizing the poultry bird birds with known complete adjuvant, each ml of said adjuvant comprising heat killed and dried 1 mg of Mycobacterium tuberculosis (H37Ra, ATCC 25177), 0.85 ml paraffin and 0.15 ml mannide monooleate;
- (c) immunizing the bird birds with 1000 μ g of ~~conjugate selected from DDT-OH hapten, Octachloro cyclic hapten,~~ the hapten protein conjugate 2,4,5 trichlorophoxyacetic acid β -alanine ~~in~~ administered to breast muscle;
- (d) immunizing the bird birds again with the hapten-protein conjugate as given in step (c) with 500 μ g of ~~desired~~ the hapten conjugate;
- (e) immunizing the bird birds with the hapten-protein conjugate at the intervals of two, three and five weeks;
- (f) immunizing the bird birds thereafter with the hapten-protein conjugate at five weeks intervals as long as the bird lays eggs; and
- (g) harvesting antibodies from the egg yolk of the bird birds.

2. (Currently amended) A process as claimed in claim 1, wherein the hapten protein conjugate has binding properties to hexachlorohexane. ~~desired hapten-protein conjugates having binding properties to DDT, Endosulphan and Hexachlorohexane.~~

3. (Canceled)

4. (Canceled)

5. (Currently amended) A process as claimed in claim 1, wherein the production of conjugate hapten 2,4,5-trichloro ~~Trichloro~~ phenoxy acetic acid β -alanine (TCB) hapten binding to hexachloro ~~Hexachloro~~ hexane, as per step(c), is as follows:

(a) adding ~~of~~ β -alanine spacer arm to 2,4,5-trichlorophenoxyacetic ~~Trichlorophenoxyacetic~~ acid by suspending 10 mM, 2.55 g of 2,4,5-trichlorophenoxyacetic ~~Trichlorophenoxyacetic~~ acid in 5.95 ml thionyl chloride (~~9-50 mmol~~);

(b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation;

(c) stirring the product with β -alanine in 1M NaOH (~~9 mmol, 0.66 g in 7.4 ml of 1M NaOH~~) at 0 ° C;

(d) warming the product for over 16 hours at room temperature;

(e) isolating the resulting acid by acidification;

(f) partitioning into ethyl acetate;

(g) washing with water and brine;

(h) giving an yield of crude product hapten containing 2,4,5-trichlorophenoxyacetic ~~Trichlorophenoxyacetic~~ acid (2,4,5-T) as impurity;

(i) dissolving the impurity in acetone to obtain colorless flakes of the trichlorobenzene ~~Trichlorobenzene~~(TCB) hapten;

(j) filtering and washing the colourless TCB hapten with acetone and drying in air;

(k) using silica gel precoated aluminum plates and a mixture of chloroform and methanol in a ratio of 85:15 as eluent showed a single spot in TLC analysis Rf-0.45 detected by spraying with 2% o-tolidine in acetone and exposure to UV light or sunlight ~~Uv-light/sunlight~~, at a melting range of 169-70 ° C; ~~169-70 ° C~~.

(l) synthesizing the active ester of hapten 2,4,5-T- β -alanine at melting range of 102-104 ° C by dissolving in dichloromethane; ~~dichloromethane~~.

(m) adding N- and the mixture is cooled in an ice-bath;

(n) adding dimethylsulphoxide hydroxysuccinimide ~~Dimethylsulphoxide~~(DMSO) dropwise to the mixture until the hapten is dissolved;

- (o) adding dicyclohexylcarbodiimide ~~Dicyclohexylcarbodiimide~~ to the mixture followed by adding dimethylaminopyridine catalyst;
- (p) stirring the mixture overnight and allowing the temperature to slowly raise ~~raised~~ to the room temperature;
- (q) filtering and evaporating the acetone; and
- (r) separating the active ester as a colorless solid.

6. (Currently amended) A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, is as follows:

- (a) obtaining egg yolk ~~egg yolk~~ without rupturing the yolk;
- (b) adding 100 ml of Tris buffer saline for every 10 ml of yolk;
- (c) removing the precipitate by centrifugation;
- (d) adding to the supernatant the precipitating solution of magnesium chloride and phosphotungstic acid for centrifuging;
- (e) discarding the pellet;
- (f) adding to the supernatant a water soluble protein fraction 12% polyethylene glycol;
- (g) incubating for 10 minutes and then centrifuging again;
- (h) precipitating out the antibody;
- (i) adding 10 ml of 10 mM phosphate buffer to dissolve the precipitate;
- (j) cooling the antibody solution 0 ° C; ~~0 ° C~~;
- (k) adding 10 ml of pre-cooled ethanol;
- (l) centrifuging the solution at 4 ° C and dissolving the sediment in 10 mM phosphate buffer; and
- (m) dialyzing against phosphate buffer for 24 h at 4 ° C. to obtain the yield of antibodies.

7. (Currently amended) A process as claimed in claim 1, wherein harvesting of antibodies as defined in step(g) of claim 1, can also be conducted as follows:

- (a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane;
- (b) adding for every 10 ml of yolk, 10 ml of distilled water;

- (c) adding about 0.15% of kappa-carrageenan ~~kappa-carragenanin~~ and left to stir for 30 minutes at room temperature;
- (d) filtering and centrifuging the solution at for 15 minutes;
- (e) passing through the DEAE-sephacel column prepared with 20 mM phosphate buffer pH 8.0;
- (f) eluting with 0.2 M phosphate buffer pH 8.0;
- (g) collecting the eluate and the absorbance read at 280 nm; and
- (h) pooling and storing the peak fractions containing the antibody at 4 ° C.

8. (Currently amended) A process as claimed in claim 6, wherein the lipid from the egg yolk is precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride and centrifuged and an ~~obtaining the~~ antibody yield of up to 75% is obtained from the supernatant.

9. (Currently amended) A process as claimed in claim 6, wherein the pH of the water soluble protein fraction obtained after the removal of the lipids is adjusted to pH 5.0 to further precipitate out the antibodies for obtaining a yield of 80-90%.

10. (Currently amended) A process as claimed in claim 7, wherein the yield of antibody is up to ~~the extent of~~ 73%.

11. (Currently amended) A process as claimed in claim 1, wherein the hyper immune eggs are collected daily and stored 4 ° C ~~40 ° C~~ until further use.

12. (Currently amended) A process as claimed in claim 1, wherein commencing the production of the antibody from the seventh ~~[[7th]]~~ day after the immunization and continued for 60 days.

13. (Original) A process as claimed in claim 1, wherein the titer of the antibody produced is 165-225 mg/ml.

14. (Currently amended) A process as claimed in claim 1, wherein production of the egg yolk antibody is more or equally ~~more/equally~~ sensitive to the polyclonal or monoclonal ~~polyclonal/monoclonal~~ antibodies produced using mammals.

15. (Currently amended) A process as claimed in claim 1, wherein ~~production of~~ the egg yolk antibodies produced bind ~~relates to~~ small molecule organic pesticides. ~~molecules of pesticides.~~